GLUCAN BINDING PROTEIN AND GLUCOSYLTRANSFERASE IMMUNOGENS

RELATED APPLICATIONS

This application is a continuation-in-part of U.S. Application No. 10/383,930, filed March 7, 2003, which claims the benefit of U.S. Provisional Application No. 60/363,209, filed March 7, 2002 and U.S. Provisional Application 60/402,483, filed August 8, 2002; this application is also a continuation-in-part of U.S. Application No. 09/290,049, filed April 12, 1999, which claims the benefit of U.S. Provisional Application No. 60/081,550, filed April 13, 1998 and U.S. Provisional Application No. 60/115,142, filed January 8, 1999, the entire contents of each are hereby incorporated by reference.

GOVERNMENT SUPPORT

The invention was supported, in whole or in part, by a grant DE-06153 from National Institute for Dental Research. The Government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Mutans streptococci have been implicated in the initiation of dental caries in humans. *Streptococcus mutans* have several virulence factors that allow the bacteria to accumulate within the dental biofilm and produce and tolerate the acids that cause dental caries. The ability of cariogenic *mutans streptococci* to accumulate in the dental biofilm is thought to be a consequence of the synthesis of glucans by glucosyltransferases, followed by the binding of the bacteria to these polymers via the cell-associated glucan binding proteins (Gbps). Biofilm development occurs in two distinct phases. During the first phase, bacterial surface proteins interact with host or bacterial products adsorbed on the tooth surface. In the second phase, a biofilm forms as bacteria accumulate by aggregation with the same or other species and produce an extracellular polysaccharide matrix.

Epitopes associated with these functions are thought to be primary targets for immunogenic attack, provided that the relevant sequences are located in molecular areas that can be accessible to antibody. Several *mutans streptococcal* proteins with glucan binding activity have been described (Russell, R.R., *J. Gen. Microbiol.*, 112:197-201 (1979); Smith D. J. *et al., Infect. Immun.* 62:2545-2552 (1994); Sato, Y., *et al., Infect. Immun.*, 65:668-675 (1997)). One of these components, glucan-binding protein- B (GbpB), has been shown to induce protective immune responses against experimental dental caries following systemic or

mucosal immunization (Smith D. J. *et al., Infect. Immun.* 64:3069-3073 (1996) and Smith D. J. *et al.*, Oral Microbiol. Immunol. 13:278-285(1998)). Furthermore, there is evidence that the expression of GbpB is directly related to biofilm formation (Mattos-Graner, R.O., *et al., Infect. and Immun.* 69(11) 6931-6941(2001)). However, use of the intact GbpB protein in a vaccine may induce immunity to irrelevant or unwanted epitopes.

SUMMARY OF THE INVENTION

The invention provides improved immunogens and vaccine compositions for inducing antibody production against Streptococcal antigens. Accordingly, the invention features a composition containing a fragment of a glucan binding protein-B (GbpB), which binds to a major histocompatibility complex (MHC) class II protein, e.g., an HLA protein selected from the group consisting of DRA, DRB1, DRB2, DQA1, DQB1, DPA1, DPB1, DMA, DMB, DOA, and DOB. The GbpB protein is preferably derived from a *Streptococcus mutans* strain. For example, the Streptococcal GbpB contains an amino acid sequence selected from the group consisting of SEQ ID NO's: 29, 30, 31, 32, and 33. Preferably, the GbpB protein contains the amino acid sequence of SEQ ID NO: 29 (*S. mutans* strain SJ32).

The fragment is greater than 6 and less than 431 residues in length. For example, the fragment is less than 400 residues in length, less than 100 residues in length, or less than 50 residues in length. Preferably, the fragment is 10-25 residues in length. The fragment contains an amino acid sequence selected from the group consisting of SEQ ID NO's: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, and 22. Preferably, the fragment contains an amino acid sequence selected from the group consisting of SEQ ID NO's: 1 and 3.

Also within the invention is a chimeric polypeptide containing a fragment of two or more streptococcal proteins. For example, the composition contains a GbpB polypeptide and a glucosyltransferase (GTF) polypeptide. The polypeptides are covalently linked. The chimeric polypeptide contains greater than two epitopes (diepitopic polypeptide), and may contain 3, 4, 5 or more epitopes (multiepitopic polypeptide). Optionally, the polypeptide contains two or more copies of a single epitope. The GbpB polypeptide preferably contains an amino acid sequence selected from the group consisting of SEQ ID NO's: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, and 22, and the glucosyltransferase polypeptide comprises a catalytic domain of SEQ ID NO: 34, 35, 36, 37, 38, 39, or 40. Preferably, the catalytic domain contains an amino acid sequence of SEQ ID NO: 24 or 25.

Alternatively (or in addition), the glucosyltransferase polypeptide contains a glucan binding domain of SEQ ID NO: 34, 35, 36, 37, 38, 39, or 40. Preferably, the glucan binding domain comprises an amino acid sequence of SEQ ID NO: 23, and the glucosyltransferase polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 23, 24, 25, 26, 27, AND 28. For example, a diepitopic polypeptide construct includes a GbpB polypeptide containing SEQ ID NO: 1 and a glucosyltransferase polypeptide containing SEQ ID NO: 23 or a GbpB polypeptide containing SEQ ID NO: 1 and a glucosyltransferase polypeptide containing SEQ ID NO: 25. The di- or multi-epitopic constructs optionally contain a peptidyl core matrix. The matrix contains one or a plurality of lysine residues.

The compositions are used to elicit production of an antibody in a mammal. The method is carried out by administering to the mammal a composition containing a MHC class II-binding fragment of GbpB or a composition containing both a GbpB polypeptide and a glucosyltransferase polypeptide. In the latter case, the amount of an anti-GbpB antibody produced by the mammal is at least 10% greater than an amount produced by a mammal immunized with a composition comprising a GbpB peptide in the absence of a GTF peptide. Anti-GbpB titers in animals immunized with a di or multi-epitopic peptide constructs are preferably at least 20%, at least 50%, at least 75%, and at least 100% greater than titers achieved in animals immunized with a mono-epitopic peptide. Similarly, anti-GTF titers in animals immunized with a di or multi-epitopic peptide constructs are preferably at least 20%, at least 50%, at least 75%, and at least 100% greater than titers achieved in animals immunized with a mono-epitopic peptide. The immunization leads to production of mucosal immunity (IgA isotype) as well as systemic immunity (e.g., IgG isotype). Also, within the invention is a substantially pure antibody produced by any of the methods described above.

The polypeptides (including antibody molecules) within the invention are substantially pure. A polypeptide is substantially pure when it is separated from those contaminants, which accompany it in its natural state (proteins and other naturally-occurring organic molecules). In the case of an antibody preparation, the antibodies are purified from other blood components such as cells and other blood proteins. Typically, the polypeptide is substantially pure when it constitutes at least 60%, by weight, of the protein in the preparation. Preferably, the protein in the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, of the desired protein. Purity is measured by any appropriate method, e.g., column chromatography, polyacrylamide gel

electrophoresis, or HPLC analysis. Accordingly, substantially pure polypeptides include synthetic polypeptides, recombinant polypeptides derived from a eucaryote but produced in *E. coli* or another procaryote, or in a eucaryote other than that from which the polypeptide was originally derived.

The peptides are prepared synthetically or by recombinant DNA technology. The term peptide is used interchangeably with polypeptide in the present specification to designate a series of amino acids connected one to the other by peptide bonds between the alpha-amino and alpha-carboxy groups of adjacent amino acids. Optionally, one or more peptide bonds are replaced with an alternative type of covalent bond (a "peptide mimetic") which is less susceptible to cleavage by peptidases compared to a peptide bond. Where proteolytic degradation of the peptides following injection into the subject is a problem, replacement of a particularly sensitive peptide bond with a noncleavable peptide mimetic yields a peptide mimetic, which is more stable and thus more useful as a therapeutic. Such mimetics, and methods of incorporating them into peptides, are well known in the art. Similarly, the replacement of an L-amino acid residue is a standard way of rendering the peptide less sensitive to proteolysis. Also useful are amino-terminal blocking groups such as t-butyloxycarbonyl, acetyl, theyl, succinyl, methoxysuccinyl, suberyl, adipyl, azelayl, dansyl, benzyloxycarbonyl, fluorenylmethoxycarbonyl, methoxyazelayl, methoxyadipyl, methoxysuberyl, and 2,4,-dinitrophenyl. The polypeptides or peptides are either in their neutral (uncharged) forms or in forms which are salts, and either free of modifications such as glycosylation, side chain oxidation, or phosphorylation or containing these modifications, subject to the condition that the modification not destroy the immune stimulatory activity of the polypeptides.

Derivative peptide epitopes have an amino acid sequence, which differs from the amino acid sequence of a naturally-occurring receptor peptide. Such derivative peptides have at least 50% identity compared to a reference sequence of amino acids, e.g., a naturally-occurring glutamate receptor peptide. Preferably, a derivative is 90, 95, 98, or 99% identical to a naturally-occurring protein sequence. The derivative contains a conservative amino acid substitution. By conservative substitution is meant a replacement of an amino acid residue with another, which is biologically and/or chemically similar, e.g., one hydrophobic residue for another, or one polar residue for another. The substitutions include combinations such as Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr. Nucleotide and amino acid comparisons described herein are carried out using the Lasergene software

package (DNASTAR, Inc., Madison, WI). The MegAlign module used is the Clustal V method (Higgins et al., 1989, CABIOS 5(2):151-153). The parameter used is gap penalty 10, gap length penalty 10.

In addition to eliciting active immunity by immunizing a mammal with streptococcal immunogens, the method encompasses methods of conferring passive immunity. For example, antibodies produced *in vitro* or *in vivo* are purified and administered to a mammal. The antibody preparation contains antibodies, which specifically bind to streptococcal antigens such as GbpB and/or GTF. For example, the antibodies used in a passive immunization regimen were raised by immunization of a first mammal with a composition containing a purified antibody which specifically binds to an MHC class II binding fragment of GbpB or one or more of the multi-epitopic constructs described above. Following purification of the antibodies from the first animal, the antibodies are administered to a second animal. Alternatively, antibodies are produced in culture, purified, and administered to a mammal to confer passive immunity. Antibodies elicited by immunization or administered passively inhibit one or more activities, e.g., colonization, of oral Streptococci.

Other features and advantages of the invention will be apparent from the following detailed description and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a chart depicting the results of an MHC class motif-matching algorithm used to compare GbpB primary sequence against a set of DBRI alleles represented as matches versus sequence.

FIG. 2 depicts box plots of serum IgG antibody to GbpB peptide constructs QGQ and SYI. Serum IgG antibody activity was measured in ELISA against QGQ (left panel) and SYI (middle panel) peptide constructs and GbpB protein (right panel). Sham-immunized and SYI, QGQ- and GbpB-immunized groups represent the immune experience of 6 rats per group 35 days after the initial of two subcutaneous immunizations. The absorbency was measured at 405nm.

FIG. 3 depicts box plots of serum IgG and IgA antibody to SYI in the protection experiment. IgG (left panel) and IgA (right panel) antibody activity was measured in ELISA against SYI in sera collected at the end of the protection experiment. Sham-immunized and SYI-immunized groups represent the immune experience of 13 rats per group three months

after the initial of two subcutaneous immunizations. The absorbency was measured at 405nm.

FIG. 4 depicts box plots of serum IgG and IgA antibody to GbpB in the protection experiment. IgG (left panel) and IgA (right panel) antibody activity was measured in ELISA against GbpB in sera collected at the end of the protection experiment. Sham-immunized and SYI-immunized groups represent the immune experience of 13 rats/per group three months after the initial of two subcutaneous immunizations. The absorbency was measured at 405nm.

FIG. 5 depicts the level of infection after challenge with *S. mutans*. Each plot represents the number of *S. mutans* SJr cultivated after systematic swabbing of molar surfaces eight days and 65 days after initial infection with *S. mutans* SJr. Bars indicate the mean colony forming units of *S. mutans* SJr in sham- or SYI-immunized groups. Open and closed circles indicate levels of infection of individual rats.

FIG. 6 depicts dental caries after 78 days of infection with *S. mutans* SJr. The buccal, lingual, occlusal and total molar caries scores for sham and SYI-immunized groups are shown in respective panels.

FIG. 7 is a box plot showing serum IgG antibody binding to SYI peptide depicted as absorbency units at 405nm as measured in an ELISA assay. Sera was collected on day 63.

FIG. 8 is a box plot showing serum IgG antibody binding to *S. mutans* GbpB depicted as absorbency units at 405nm as measured in an ELISA assay. Sera was collected on day 63.

FIG. 9 is a box plot showing serum IgG antibody binding to GLU peptide depicted as absorbency units at 405nm as measured in an ELISA assay. Sera was collected on day 63.

FIG. 10 is a box plot showing serum IgG antibody binding to CAT peptide depicted as absorbency units at 405nm as measured in an ELISA assay. Sera was collected on day 63.

FIG. 11 is a box plot showing serum IgG antibody binding to *S. sobrinus* GTF depicted as absorbency units at 405nm as measured in an ELISA assay. Sera was collected on day 63.

FIG. 12 is a pictorial representation of the peptide and protein antigens used for the immunization of rats in the ELISA binding assays (results of which are shown in FIGS. 7-11).

DETAILED DESCRIPTION OF THE INVENTION

Mutans streptococcus is the principle etiologic agent of the infectious disease dental caries. This oral pathogen infects the oral cavity during early childhood and normally remains associated with the host's dentition for life. The accumulation of bacteria within the dental biofilm is possible due to the effect of several virulence factors. The bacterial components associated with the accumulation phase of mutans streptococci include glucosyltransferases, their glucan products and glucan binding proteins. At least three *S. mutans* glucan binding proteins have been identified, GbpA, GbpB and GbpC. GbpA shares homology with the putative glucan binding domain of glucosyltransferase and the gbpA gene was found to encode a constitutively expressed secreted protein. Cell surface associated GbpC is related to the Spa family of streptococcal proteins and is only expressed during conditions of stress. GbpB is immunogenically distinct from the other glucan binding proteins expressed by *S. mutans* and *Streptococcus sobrinus* and also differs in size and purification properties.

Glucan binding protein-B is a single polypeptide chain, which is 431-432 residues in length. Analysis of the primary sequence revealed a leucine zipper domain. However, GbpB bore no sequence homology with glucan binding domains of glucosyltransferases or *S. mutans* glucan binding protein A. This prevented the specific targeting of GbpB domains of putative glucan binding function using subunit vaccine approaches that had been employed successfully with synthetic peptide or recombinant construct derived from GTF glucan binding domains as described in U.S. Patent No. 5,686,075 and U.S. Application No. 09/290,049, the entire contents of which are herein incorporated by reference.

The GbpB sequence bears significant homology with peptidoglycan hydrolases from other gram positive microorganisms, and comparative genomic analysis of the *gbpB* region suggested a functional relationship between genes involved in cell shape and cell wall maintenance. Attempts to knock out the *gbpB* gene indicated that expression of GbpB is essential for the organism. Immunogenic interference with GbpB function reduces the adverse effects associated with the growth of cariogenic *S. mutans* in the oral cavity.

Tables 5-9 include the amino acid sequence of GbpB from various strains *S. mutans*.

Table 5
Deduced Amino Acid Sequence of *S. mutans* strain SJ32 GbpB
GenBank accession number AY046410

MKKRILSAVLVSGVTLSSATTLSAVKADDFDAQIASQDSKINNLTAQQQA
AQAQVNTIQGQVSALQTQQAELQAENQRLEAQSATLGQQIQTLSSKIVAR
NESLKQQARSAQKSNAATSYINAIINSKSVSDAINRVSAIREVVSANEKM
LQQQEQDKAAVEQKQQENQAAINTVAANQETIAQNTNALNTQQAQLEAAQ
LNLQAELTTAQDQKATLVAQKAAAEEAARQAAAAQAAAEAKAAAEAKALQ
EQAAQAQVAANNNTQATDASDQQAAAADNTQAAQTGDSTEQSAAQAVNNS
DQESTTATEAQPSASSASTAAVAANTSSANTYPAGQCTWGVKSLAPWVGN
YWGNGGQWAASAAAAGYRVGSTPSAGAVAVWNDGGYGHVAYVTGVQGGQI
QVQEANYAGNQSIGNYRGWFNPGSVSYIYPN (SEQ ID NO:29)

Table 6
Deduced Amino Acid Sequence of *S. mutans* strain 3VF4 GbpB
GenBank accession number AY046411

MKKRILSAVLVSGVTLSSATTLSAVKADDFDAQIASQDSKINNLTAQQQA
AQAQVNTIQGQVSALQTQQAELQAENQRLEAQSATLGQQIQTLSSKIVAR
NESLKQQARSAQKSNAATSYINAIINSKSVSDAINRVSAIREVVSANEKM
LQQQEQDKAAVEQKQQENQAAINTVAANQETIAQNTNALNTQQAQLEAAQ
LNLQAELTTAQDQKATLVAQKAAAEEAARQAAAAQAAAEAKAAAEAKALQ
EQAAQAQAAANNNTQATDASDQQAAAADNTQAAQTGDSTEQSAAQAVNNS
DQESTTATEAQPSASSASTAAVAANTSSANTYPAGQCTWGVKSLAPWVGN
YWGNGGQWAASAAAAGYRVGSTPSAGAVAVWNDGGYGHVAYVTGVQGGQI
QVQEANYAGNQSIGNYRGWFNPGSVSYIYPN (SEQ ID NO:30)

Table 7
Deduced Amino Acid Sequence of *S. mutans* strain 15JP2 GbpB
GenBank accession number AY046412

MKKRILSAVLVSGVTLSSATTLSAIKADDFDAQIASQDSKINNLTAQQQA
AQAQVNTIQGQVSALQTQQAELQAENQRLEAQSATLGQQIQTLSSKIVAR
NESLKQQARSAQKSNAATSYINAIINSKSVSDAINRVSAIREVVSANEKM
LQQQEQDKAAVEQKQQENQAAINTVAANQETIAQNTNALNTQQAQLEAAQ
LNLQAELTTAQDQKATLVAQKAAAEEAARQAAAAQAAAEAKAAAEAKALQ
EQAAQAQAAANNNNTQATDASDQQAAAADNTQAAQTGDSTDQSAAQAVNN
SDQESTTATAAQPSASSASTAAVAANTSSANTYPAGQCTWGVKSLAPWVG
NYWGNGGQWAASAAAAGYRVGSTPSAGAVAVWNDGGYGHVAYVTGVQGGQ
IQVQEANYAGNQSIGNYRGWFNPGSVSYIYPN (SEQ ID NO:31)

Table 8
Deduced Amino Acid Sequence of *S. mutans* strain 3SN1 GbpB
GenBank accession number AY046413

MKKRILSAVLVSGVTLSSATTLSAVKADDFDAQIASQDSKINNLTAQQQA
AQAQVNTIQGQVSALQTQQAELQAENQRLEAQSATLGQQIQTLSSKIVAR
NESLKQQARSAQKSNAATSYINAIINSKSVSDAINRVSAIREVVSANEKM
LHQQEQDKAAVEQKHQENQAAINTVAANQETIAQNTNALNTQQAQLEAAQ
LNLQAELTTAQDQKATLVAQKAAAEEAARQAAAAQAAAEAKAAAEAKALQ
EQAAQAQAAANNNNTQATDASDQQAAAADNTQAAQTGDSTDQSAAQAVNN
SDQESTTATAAQPSASSASTAAVAANTSSANTYPAGQCTWGVKSLAPWVG
NYWGNGGQWAASAAAAGYRVGSTPSAGAVAVWNDGGYGHVAYVTGVQGGQ
IQVQEANYAGNQSIGNYRGWFNPGSVSYIYPN (SEQ ID NO:32)

Table 9
Deduced Amino Acid Sequence of *S. mutans* strain 5SM3 GbpB
GenBank accession number AY046414

MKKRILSAVLVSGVTLSSATTLSAVKADDFDAQIASQDSKINNLTAQQQA
AQAQVNTIQGQVSALQTQQAELQAENQRLEAQSATLGQQIQTLSSKIVAR
NESLKQQARSAQKSNAATSYINAIINSKSVSDAINRVSAIREVVSANEKM
LQQQEQDKAAVEQKQQENQAAINTVAANQETIAQNTNALNTQQAQLEAAQ
LNLQAELTTAQDQKATLVAQKAAAEEAARQAAAAQAAAEAKAAAEAKALQ
EQAAQAQAAANNNTQATDASDQQAAAADNTQAAQTGDSTEQSAAQAVNNS
DQESTTATEAQPSASSASTAVVTANTSSANTYPAGQCTWGVKSLAPWVGN
YWGNGGQWAASAAAAGYRVGSTPSAGAVAVWNDGGYGHVAYVTGVQGGQI
QVQEANYAGNQSIGNYRGWFNPGSVSYIYPN (SEQ ID NO:33)

The compositions described herein, *e.g.,* subunit vaccine compositions and immunogenic compositions, contain an amino acid sequence subunit of GbpB that is of sufficient length to raise an immune response in a mammal to which it is administered. As used herein, the terms "subunit" or "fragment" refer to a portion of the GbpB protein that is less than the whole naturally-occurring protein. For example, a fragment contains at least 5 contiguous amino acids of the full length naturally-occurring protein. Vaccines containing the peptide constructs described herein elicit antibodies, which bind specifically to functional domains of GbpB and/or GTF and have the additional advantage that such vaccines do not induce immunity to irrelevant or unwanted epitopes. Useful peptides are of sufficient length to raise an immune response in a mammal to which it is administered but will be less than the complete amino acid sequence of the intact GbpB. Typically, the peptide is at least 5-7 amino acids in length. Preferably the peptide is at least 12 amino acids in length; more preferable the peptide is at least 23 amino acids in length. GbpB polypeptides are derived from *S. mutans*. However, glucan binding proteins from the other strains of *S. mutans*, which

share significant homology and/or function, can also be utilized. For example, a peptide in the immunogenic compositions and subunit vaccines of the invention typically comprise at least six amino acids with at least four matches to MHC Class II binding motifs. Preferably, the peptide has greater than five amino acid matches and most preferably the peptide has greater than six amino acid matches to an MHC class II binding motif. The matches are determined, for example, using a matrix-based algorithm for epitope prediction known in the art.

Peptides as shown in FIG.1 which have a significant peak resulting from a comparison of GbpB primary sequence against a set of DBRI alleles from MHC class II motif are also contemplated. For example, according to FIG. 1, peptides which extend at least 6 amino acid residues to the right in length from residues 16, 62, 90, 121, 322 and 369 are peptides having at least four matching residues to DBR1 alleles and are contemplated for use in the compositions of the instant application. Suitable peptides may also encompass amino acid residues to the left of the indicated peak.

HLA-binding peptides of GbpB

GbpB peptides were synthesized and evaluated for immunogenicity, reactivity with the parent protein, and induction of caries-protective immunity. Exemplary peptides include the following fragments of glucan binding protein-B: KSNAATSYINAIINSKSVSD (the SYI peptide GbpB residues 113-132) (SEQ ID NO: 1); KHKLITIQGQVSALQTQQAG (SEQ ID NO: 2); the SAS peptide TATEAQPSASSASTAAVAAN residues 306-325 (SEQ ID NO: 3); LSAVLVSGVTLSSATTLSAV residues 6-25 (SEQ ID NO: 4); LSSATTLSAVKADDFDAQIA residues 16-35 (SEQ ID NO: 5); QIASQDSKINNLTAQQQAAQ residues 33-52 (SEQ ID NO: 6); QDSKINNLTAQQQAAQAQVN residues 37-56 (SEQ ID NO: 7); QQAAQAQVNTIQGQVSALQT residues 48-67 (SEQ ID NO: 8); QAQVNTIQGQVSALQTQQAE residues 52-71 (SEQ ID NO: 9); QQIQTLSSKIVARNESLKQQ residues 88-107 (SEQ ID NO: 10); ATSYINAIINSKSVSDAINR residues 117-136 (SEQ ID NO: 11); VSAIREVVSANEKMLQQQEQ residues 137-156 (SEQ ID NO: 12); TVAANQETIAQNTNALNTQQ residues 174-193 (SEQ ID NO: 13); AQLEAAQLNLQAELTTAQDQ residues 194-213 (SEQ ID NO: 14); KATLVAQKAAAEEAARQAAA residues 214-233 (SEQ ID NO: 15); ALQEQAAQAQVAANNNTQAT residues 248-267 (SEQ ID NO: 16);

TEQSAAQAVNNSDQESTTAT residues 289-308 (SEQ ID NO: 17);
QPSASSASTAAVAANTSSAN residues 311-330 (SEQ ID NO: 18);
GNYWGNGGQWAASAAAAGYR residues 349-368 (SEQ ID NO: 19);
AGYRVGSTPSAGAVAVWNDG residues 365-384 (SEQ ID NO: 20);
DGGYGHVAYVTGVQGGQIQV residues 383-402 (SEQ ID NO: 21);
QEANYAGNQSIGNYRGWFNP residues 403-422 (SEQ ID NO: 22);
GNYWGNGGQWAASAAAAGRY (SEQ ID NO: 41). Amino acid residue coordinates refer to full length GbpB (SEQ ID NO:29). Equivalent peptides are intended to include equivalent sites (*e.g.*, positions or residues) in other mutans streptococcal glucan binding proteins. For example, other glucan binding peptides can be found in *S. sobrinus* or other *S. mutans* strains. The equivalents can be identified, for example, by aligning the amino acid sequences of other mutans streptococcal GbpB's, as is routinely done by one of skill in the art.

As used herein, a vaccine composition is a composition, which elicits an immune response in a mammal to which it is administered. Elicitation of GbpB-specific antibodies protects the immunized mammal against subsequent challenge by the immunizing agent or an immunogenically cross-reactive agent. For example, production of mucosal antibodies specific for GbpB reduces the amount of *S. mutans* in an immunized mammal. Protection can be complete or partial, such as a reduction or elimination of symptoms or infection as compared with an unvaccinated mammal. An immunogenically cross-reactive agent can be, for example the whole protein (GbpB) from which a subunit peptide used as the immunogen is derived. Alternatively, an immunogenically cross-reactive agent can be a different protein, which is recognized in whole or in part by the antibodies elicited by the immunizing agent.

As used herein, an immunogenic composition encompasses a composition, which elicits an immune response in a mammal to which it is administered and which may or may not protect the immunized mammal against subsequent challenge with the immunizing agent or an immunogenically cross-reactive agent.

The raised immune response is characterized by a B cell response, a T cell response or both a B cell and T cell response. The B cell response is associated with the appearance of mucosal antibody, which is predominately IgA, and systemic antibody, which is predominantly IgG. The antibodies elicited by immunization preferably recognize both the immunizing agent and an immunogenically cross-reactive agent (*e.g.*, the immunizing peptide and the intact GbpB protein). The antibody response protects the immunized

mammal against subsequent challenge or infection with the immunizing agent or an immunogenically cross-reactive agent.

In addition to the peptides listed in Table 1, other immunogenic domains of GbpB, as well as domains of non-GbpB origin, which enhance adjuvanticity or produce an immunogenic response against other infectious agents are optionally included in the compositions of the invention. For example, the vaccine or immunogenic composition contains an additional immunogenic component which is an immunogenic portion of a pathogen including, but not limited to, diphtheria, pertussis, tetanus, measles, influenza, poliovirus, and retroviruses resulting in a multivalent composition raising an immune response to greater than one infectious disease or agent. A multivalent vaccine includes immunogenic epitopes and appropriate adjuvant sequences targeting early childhood infections.

GbpB-GTF Chimeric Peptides

Immunogenic compositions containing one or more domains of GbpB combined with one or more domains of GTF were produced and evaluated for the ability to elicit antibody production. This strategy permits a combined attack on the molecular pathogenesis of *mutans streptococci* utilizing two or more epitopes. Synthetic peptides containing an amino acid sequence of one or more functional domains of *Streptococcus mutans* glucosyltransferases induce immune responses, which reduce recolonization of the bacteria. Chimeric peptides containing GTF sequences and GbpB sequences provide a more comprehensive attack on the colonization of the bacteria by increasing the enzyme inhibitory capacity of the immune response, eliminating responses to irrelevant epitopes and reducing the glucan binding capacity.

Tables 10-16 include the amino acid sequence of GTF isozymes from various *Streptococci.*

Table 10

Deduced Amino Acid Sequence of *S. mutans* GTF-B

MDKKVRYKLRKVKKRWVTVSVASAVMTLTTLSGGLVKADSNESK
SQISNDSNTSVVTANEESNVITEATSKQEAASSQTNHTVTTSSSSTSVVNPKEVVSNP
YTVGETASNGEKLQNQTTTVDKTSEAAANNISKQTTEADTDVIDDSNAANLQILEKLP
NVKEIDGKYYYYDNNGKVRTNFTLIADGKILHFDETGAYTDTSIDTVNKDIVTTRSNL
YKKYNQVYDRSAQSFEHVDHYLTAESWYRPKYILKDGKTWTQSTEKDFRPLLMTWWPD
QETQRQYVNYMNAQLGINKTYDDTSNQLQLNIAAATIQAKIEAKITTLKNTDWLRQTI
SAFVKTQSAWNSDSEKPFDDHLQNGAVLYDNEGKLTPYANSNYRILNRTPTNQTGKKD
PRYTADNTIGGYEFLLANDVDNSNPVVQAEQLNWLHFLMNFGNIYANDPDANFDSIRV
DAVDNVDADLLQIAGDYLKAAKGIHKNDKAANDHLSILEAWSDNDTPYLHDDGDNMIN
MDNKLRLSLLFSLAKPLNQRSGMNPLITNSLVNRTDDNAETAAVPSYSFIRAHDSEVQ
DLIADIIKAEINPNVVGYSFTMEEIKKAFEIYNKDLLATEKKYTHYNTALSYALLLTN
KSSVPRVYYGDMFTDDGQYMAHKTINYEAIETLLKARIKYVSGGQAMRNQQVGNSEII
TSVRYGKGALKATDTGDRTTRTSGVAVIEGNNPSLRLKASDRVVVNMGAAHKNQAYRP
LLLTTDNGIKAYHSDQEAAGLVRYTNDRGELIFTAADIKGYANPQVSGYLGVWVPVGA
ALIKMFALRLARPHQQMASVHQNAALDSRVMFEGFSNFQAFATKKEEYTNVVIAKNVD
KFAEWGVTDFEMAPQYVSSTDGSFLDSVIQNGYAFTDRYDLGISKPNKYGTADDLVKA
IKALHSKGIKVMADWVPDQMYAFPEKEVVTATRVDKYGTPVAGSQIKNTLYVVDGKSS
GKDQQAKYGGAFLEELQAKYPELFARKQISTGVPMDPSVKIKQWSAKYFNGTNILGRG
AGYVLKDQATNTYFNISDNKEINFLPKTLLNQDSQVGFSYDGKGYVYYSTSGYQAKNT
FISEGDKWYYFDNNGYMVTGAQSINGVNYYFLSNGLQLRDAILKNEDGTYAYYGNDGR
RYENGYYQFMSGVWRHFNNGEMSVGLTVIDGQVQYFDEMGYQAKGKFVTTADGKIRYF
DKQSGNMYRNRFIENEEGKWLYLGEDGAAVTGSQTINGQHLYFRANGVQVKGEFVTDH
HGRISYYDGNSGDQIRNRFVRNAQGQWFYFDNNGYAVTGARTINGQLLYFRANGVQVK
GEFVTDRYGRISYYDGNSGDQIRNRFVRNAQGQWFYFDNNGYAVTGARTINGQHLYFR
ANGVQVKGEFVTDRHGRISYYDGNSGDQIRNRFVRNAQGQWFYFDNNGYAVTGARTIN
GQHLYFRANGVQVKGEFVTDRYGRISYYDANSGERVRIN (SEQ ID NO:34)

Table 11

Deduced Amino Acid Sequence of *S. mutans* GTF-C

MEKKVRFKLRKVKKRWVTVSIASAVVTLTSLSGSLVKADSTDDR
QQAVTESQASLVTTSEAAKETLTATDTSTATSATSQPTATVTDNVSTTNQSTNTTANT
ANFVVKPTTTSEQAKTDNSDKIITTSKAVNRLTATGKFVPANNNTAHPKTVTDKIVPI
KPKIGKLKQPSSLSQDDIAALGNVKNIRKVNGKYYYYKEDGTLQKNYALNINGKTFFF
DETGALSNNTLPSKKGNITNNDNTNSFAQYNQVYSTDVANFEHVDHYLTAESWYRPKY
ILKDGKTWTQSTEKDFRPLLMTWWPDQETQRQYVNYMNAQLGIHQTYNTATSPLQLNL
AAQTIQTKIEEKITAEKNTNWLRQTISAFVKTQSAWNSDSEKPFDDHLQKGALLYSNN
SKLTSQANSNYRILNRTPTNQTGKKDPRYTADRTIGGYEFLLANDVDNSNPVVQAEQL
NWLHFLMNFGNIYANDPDANFDSIRVDAVDNVDADLLQIAGDYLKAAKGIHKNDKAAN
DHLSILEAWSYNDTPYLHDDGDNMINMDNRLRLSLLYSLAKPLNQRSGMNPLITNSLV
NRTDDNAETAAVPSYSFIRAHDSEVQDLIRNIIRTEINPNVVGYSFTTEEIKKAFEIY
NKDLLATEKKYTHYNTALSYALLLTNKSSVPRVYYGDMFTDDGQYMAHKTINYEAIET
LLKARIKYVSGGQAMRNQQVGNSEIITSVRYGKGALKATDTGDRTTRTSGVAVIEGNN
PSLRLKASDRVVVNMGAAHKNQAYRPLLLTTDNGIKAYHSDQEAAGLVRYTNDRGELI
FTAADIKGYANPQVSGYLGVWVPVGAAADQDVRVAASTAPSTDGKSVHQNAALDSRVM
FEGFSNFQAFATKKEEYTNVVIAKNVDKFAEWGVTDFEMAPQYVSSTDGSFLDSVIQN
GYAFTDRYDLGISKPNKYGTADDLVKAIKALHSKGIKVMADWVPDQMYALPEKEVVTA
TRVDKYGTPVAGSQIKNTLYVVDGKSSGKDQQAKYGGAFLEELQAKYPELFARKQIST
GVPMDPSVKIKQWSAKYFNGTNILGRGAGYVLKDQATNTYFSLVSDNTFLPKSLVNPN
HGTSSSVTGLVFDGKGYVYYSTSGNQAKNAFISLGNNWYYFDNNGYMVTGAQSINGAN
YYFLSNGIQLRNAIYDNGNKVLSYYGNDGRRYENGYYLFGQQWRYFQNGIMAVGLTRV
HGAVQYFDASGFQAKGQFITTADGKLRYFDRDSGNQISNRFVRNSKGEWFLFDHNGVA
VTGTVTFNGQRLYFKPNGVQAKGEFIRDANGYLRYYDPNSGNEVRNRFVRNSKGEWFL
FDHNGIAVTGARVVNGHASILSLMVFRLRESSLQSVKVVSNTMILIPEMKFVIVM

(SEQ ID NO:35)

Table 12

Deduced Amino Acid Sequence of *S. mutans* GTF-D

METKRRYKMHKVKKHWVTVAVASGLITLGTTTLGSSVSAETEQQ
TSDKVVTQKSEDDKAASESSQTDAPKTKQAQTEQTQAQSQANVADTSTSITKETPSQN
ITTQANSDDKTVTNTKSEEAQTSEERTKQSEEAQTTASSQALTQAKAELTKQRQTAAQ
ENKNPVDLAAIPNVKQIDGKYYYIGSDGQPKKNFALTVNNKVLYFDKNTGALTDTSQY
QFKQGLTKLNNDYTPHNQIVNFENTSLETIDNYVTADSWYRPKDILKNGKTWTASSES
DLRPLLMSWWPDKQTQIAYLNYMNQQGLGTGENYTADSSQESLNLAAQTVQVKIETKI
SQTQQTQWLRDIINSFVKTQPNWNSQTESDTSAGEKDHLQGGALLYSNSDKTAYANSD
YRLLNRTPTSQTGKPKYFEDNSSGGYDFLLANDIDNSNPVVQAEQLNWLHYLMNYGSI
VANDPEANFDGVRVDAVDNVNADLLQIASDYLKAHYGVDKSEKNAINHLSILEAWSDN
DPQYNKDTKGAQLPIDNKLRLSLLYALTRPLEKDASNKNEIRSGLEPVITNSLNNRSA
EGKNSERMANYIFIRAHDSEVQTVIAKIIKAQINPKTDGLTFTLDELKQAFKIYNEDM
RQAKKKYTQSNIPTAYALMLSNKDSITRLYYGDMYSDDGQYMATKSPYYDAIDTLLKA
RIKYAAGGQDMKITYVEGDKSHMDWDYTGVLTSVRYGTGANEATDQGSEATKTQGMAV
ITSNNPSLKLNQNDKVIVNMGAAHKNQEYRPLLLTTKDGLTSYTSDAAAKSLYRKTND
KGELVFDASDIQGYLNPQVSGYLAVWVPVGASDNQDVRVAASNKANATGQVYESSSAL
DSQLIYEGFSNFQDFVTKDSDYTNKKIAQNVQLFKSWGVTSFEMAPQYVSSEDGSFLD
SIIQNGYAFEDRYDLAMSKNNKYGSQQDMINAVKALHKSGIQVIADWVPDQIYNLPGK
EVVTATRVNDYGEYRKDSEIKNTLYAANTKSNGKDYQAKYGGAFLSELAAKYPSIFNR
TQISNGKKIDPSEKITAWKAKYFNGTNILGRGVGYVLKDNASDKYFELKGNQTYLPKQ
MTNKEASTGFVNDGNGMTFYSTSGYQAKNSFVQDAKGNWYYFDNNGHMVYGLQQLNGE
VQYFLSNGVQLRESFLENADGSKNYFGHLGNRYSNGYYSFDNDSKWRYFDASGVMAVG
LKTINGNTQYFDQDGYQVKGAWITGSDGKKRYFDDGSGNMAVNRFANDKNGDWYYLNS
DGIALVGVQTINGKTYYFGQDGKQIKGKIITDNGKLKYFLANSGELARNIFATDSQNN
WYYFGSDGVAVTGSQTIAGKKLYFASDGKQVKGSFVTYNGKVHYYHADSGELQVNRFE
ADKDGNWYYLDSNGEALTGSQRINDQRVFFTREGKQVKGDVAYDERRLLVYR (SEQ ID NO:36)

Table 13

Deduced Amino Acid Sequence of *S. sobrinus* GTF-I

MEKNVRFKMHKVKKRWVTLSVASATMLASALGASVASADTDTAS
DDSNQAVVTGDQTTNNQATDQTSIAATATSEQSASTDAATDQASAAEQTQGTTASTDT
AAQTTTNANEAKWVPTENENQGFTDEMLAEAKNVATAESDSIPSDLAKMSNVKQVDGK
YYYYDQDGNVKKNFAVSVGDKIYYFDETGAYKDTSKVDADKSSSAVSQNATIFAANNR
AYSTSAKNFEAVDNYLTADSWYRPKSILKDGKTWTESGKDDFRPLLMAWWPDTETKRN
YVNYMNKVVGIDKTYTAETSQADLTAAAELVQARIEQKITSENNTKWLREAISAFVKT
QPQWNGESEKPYDDHLQNGALLFDNQTDLTPDTQSNYRLLNRTPTNQTGSLDSRFTYN
PNDPLGGYDFLLANDVDNSNPVVQAEQLNWLHYLLNFGSIYANDADANFDSIRVDAVD
NVDADLLQISSDYLKAAYGIDKNNKNANNHVSIVEAWSDNDTPYLHDDGDNLMNMDNK
FRLSMLWSLAKPLDKRSGLNPLIHNSLVDREVDDREVETVPSYSFARAHDSEVQDIIR
DIIKAEINPNSFGYSFTQEEIEQAFKIYNEDLKKTDKKYTHYNVPLSYTLLLTNKGSI
PRVYYGDMFTDDGQYMANKTVNYDAIESLLKARMKYVSGGQAMQNYQIGNGEILTSVR
YGKGALKQSDKGDATTRTSGVGVVMGNQPNFSLDGKVVALNMGAAHANQEYRALMVST
KDGVATYATDADASKAGLVKRTDENGYLYFLNDDLKGVANPQVSGFLQVWVPVGAADD
QDIRVAASDTASTDGKSLHQDAAMDSRVMFEGFSNFQSFATKEEEYTNVVIANNVDKF
VSWGITDFEMAPQYVSSTDGQFLDSVIQNGYAFTDRYDLGMSKANKYGTADQLVKAIK
ALHAKGLKVMADWVPDQMYTFPKQEVVTVTRTDKFGKPIAGSQINHSLYVTDTKSSGD
DYQAKYGGAFLDELKEKYPELFTKKQISTGQAIDPSVKIKQWSAKYFNGSNILGRGAD
YVLSDQVSNKYFNVASDTLFLPSSLLGKVVESGIRYDGKGYIYNSSATGDQVKASFIT
EAGNLYYFGKDGYMVTGAQTINGANYFFLENGTALRNTIYTDAQGNSHYYANDGKRYE
NGYQQFGNDWRYFKDGNMAVGLTTVDGNVQYFDKDGVQAKDKIIVTRDGKVRYFDQHN
GNAATNTFIADKTGHWYYLGKDGVAVTGAQTVGKQKLYFEANGQQVKGDFVTSDEGKL
YFYDVDSGDMWTDTFIEDKAGNWFYLGKDGAAVTGAQTIRGQKLYFKANGQQVKGDIV
KGTDGKIRYYDAKSGEQVFNKTVKAADGKTYVIGNDGVAVDPSVVKGQTFKDASGALR
FYNLKGQLVTGSGWYETANHDWVYIQSGKALTGEQTINGQHLYFKEDGHQVKGQLVTG
TDGKVRYYDANSGDQAFNKSVTVNGKTYYFGNDGTAQTAGNPKGQTFKDGSDIRFYSM
EGQLVTGSGWYENAQGQWLYVKNGKVLTGLQTVGSQRVYFDENGIQAKGKAVRTSDGK
IRYFDENSGSMITNQWKFVYGQYYYFGNDGARIYRGWN (SEQ ID NO:37)

Table 14

Deduced Amino Acid Sequence of *S. sobrinus* GTF-U

MEKKLHYKLHKVKKHWVTIAVASIGLVSLVGAGTVSAEDKVAND
TTAQATVGVDTGQDQATTNDANTNTTDTDTADQSANTNQDQAGSDQSNNQDQAKQDTA
NTDRNQADNSQTDNNQATDQATSPATDGTSVQRRDAANVATAADQEGQTAPSEQEKSA
ALSLDNVKLIDGKYYYVQADGSYKKNFAITVNGQMLYFDSDTGALSSTSTYSFSQGTT
NLVDDFSSHNKAYDSTAKSFELVNGYLTANSWYRPAGILRNGQTWEASNENDLRPVLM
SWWPDKDTQVAYVNYMNKYLSANETEVTNETSQVDLNKEAQSIQTKIEQKITSDNSTQ
WLRTAMEAFVAAQPKWNMSTENFNKGDHLQGGALLYTNSDLTPWANSDYRLLNRTPTQ
QDGTKKYFTEGGEGGYEFLLSNDVDNSNPVVQAEQLNQLHYLMNWGDIVMGDKDANFD
GVRVDAVDNVNADLLQVYSNYFKDNYKVTDSEANALAHISILEAWSLNDNQYNEDTNG
TALSIDNSSRLTSLAVLTKQPGQRIDLSNLISESVNKERANDTAYGDTIPTYSFVRAH
DSEVQTVIAKIVKEKIDTNSDGYTFTLDQLKDAFKIYNEDMAKVNKTYTHYNIPAAYA
LLLSNMESVPRVYYGDLYTDDGQYMAKKSPYYDAIATMLQGRIAYVSGGQSEEVHKVN
GNNQILSSVRYGQDLMSADDTQGTDLSRTSGLVTLVSNDPNLDLGGDSLTVNMGRAHA
NQAYRPLILGTKDGVQSYLKDSDTNIVKYTDANGNLTFTADDIKGYSTVDMSGYLAVW
VPVGAKDGQDVRVAADTNQKADGKSLKTSAALDSQVIYEGFSNFQDFANNDADYTNKK
IAENADFFKKLGITSFEMAPQYVSATDGSFLDSIIQNGYAFSDRYDLAMSKNNKYGSK
DDLANALKALHANGIQAIADWVPDQIYQLPGEEVVTAKRTNSYGNPTFDAYINNALYA
TNTKSSGSDYQAQYGGAFLDELKAKYPDMFTVNMISTGKPIDPSTKIKQWEAKYFNGT
NVLGKGAGYVLSDDATGKYFTVNENGDFLPASFTGDQNAKTGFYYDGTGMAYYSTSGN
KAVNSFIYEGGHYYYFDKDGHMVTGSYKAEDGNDYYFLPNGIQMRDAIYQDAQGNSYY
YGRTGILYKGDNWYPFVDPNNANKTVFRYFDANNVMAIGYRNMYGQTYYFDENGFQAK
GQLLTDDKGTHYFDEDNGAMAKNKFVNVGDDWYYMDGNGNAVKGQYPVNNQILYFNPE
TGVQVKGQFITDAQGRTSYYDANSGALKSSGFFTPNGSDWYYAENGYVYKGFKQVAEN
QDQWYYFDQTTGKQAKGAAKVDGRDLYFNPDSGVQVKGDFATDESGNTSFYHGDNGDK
VVGGFFTTGNNAWYYADNNGNLVKGFQEIDGKWYHFDEVTGQQAKGAALVNGQQLYFD
VDSGIQVKGDFVTDGQGNTSYYDVNSGDKKVNGFFTTGDNAWYYADGQGNLAKGRKSI
DNQDLYFDPATGKQVKGQLVSIDGRNYYFDSGSGNMAKNRFVRIGDQWIYFGNDGAAT
NL (SEQ ID NO:38)

Table 15

Deduced Amino Acid Sequence of *S. downei* GTF-S

MEKNLRYKLHKVKKQWVAIGVTTVTLSFLAGGQVVAADTNNNDG
TSVQVNKMVPSDPKFDAQAQNGQLAQAMFKAANQADQTATSQVSPATDGRVDNQVTPA
ANQPAANVANQDVANPATDAGALNRQSAADTSTDGKAVPQTSDQPGHLETVDGKTYYV
DANGQRLKNYSMVIDGKTYYFDGQTGEAQTDLPKTGQANQDNVPDSYQANNQAYSNEA
SSFETVDNYLTADSWYRPRKILKNGQSWQASSEGDLRPILMTWWPDAATKAAYANFWA
KEGLISGSYRQNSANLDAATQNIQSAIEKKIASEGNTNWLRDKMSQFVKSQNQWSIAS
ENETVYPNQDHMQGGALLFSNSKDTEHANSDWRLLNRNPTFQTGKQKYFTTNYAGYEL
LLANDVDNSNPVVQAEQLNHLHYLMNWGDIVMGDKDANFDGVRVDAVDNVNADLLQIQ
RDYYKAKYGTDQNEKNAIDHLSILEAWSGNDNDYVKDQNNFSLSIDNDQRSGMLKAFG
YASAYRGNLSNLATAGLKNRSANPDSDPVPNYVFIRAHDSEVQTRIAKIIREKLGKTN
ADGLTNLTLDDLNKAFDIYNQDMNATDKVYYPNNLPMAYAWMLQNKDTVTRVYYGDMY
TDNGQYMATKTPFYNAIETLLKGRIKYVAGGQAVSYKQDWSSGILTSVRYGKGANSAS
DAGNTETRNSGMALLINNRPNFRAYRNLTLNMGAAHKSQAYRPLLLSTKDGIATYLND
SDVDSRQYKYTDSQGNLSFSASELQSVANAQVSGMIQVWVPVGAADNQDVRTSPSTQA
TKDGNIYHQSDALDSQVIYEGFSNFQAFAQSPDQYTNAVIAKNGDLFKSWGITQFEMA
PQYVSSEDGTFLDSVILNGYAFSDRYDLAMSKNNKYGSKQDLANAIKGLQSAGIKVLS
DLVPNQLYNLPGKEVVTATRVNQYGQAKSGATINKTPYVANTRSYGDYQEQYGGKFLD
DLQKLYPRLFSTKQISTGKPIDPSVKITNWSAKYFNGSNILGRGAKYVLSEGNKYLNL
ADGKLFLPTVLNNTYGQPQVSANGFISKNGGIHYLDKNGQEVKNRFKEISGSWYYFDS
DGKMATGKTKIGNDTYLFMPNGKQLKEGVWYDGKKAYYYDDNGRTWTNKGFVEFRVDG
QDKWRYFNGDGTIAIGLVSLDNRTLYFDAYGYQVKGQTVTINGKSYTFDADQGDLVQT
DNANPAPQGQAGWKLLGDNQWGYRKDGQLLTGEQTIDGQKVFFQDNGVQVKGGTATDA
SGVLRFYDRDQGHQVGKGWYSTSDDNWVYVNESGQVLTGLQTIDGQTVYFDDKGIQAK
GKAVWDENGNLRYFDADSGNMLRDRWKNVDGNWYYFNRNGLATRW (SEQ ID NO: 39)

Table 16 Deduced Amino Acid Sequence of *S. salivarius* GTF-I
MENKIHYKLHKVKKQWVTIAVASVALATVLGGLSVTTSSVSADE TQDKTVTQSNSGTTASLVTSPEATKEADKRTNTKEADVLTPAKETNAVETATTTNTQA TAEAATTATTADVAVAAVPNKEAVVTTDAPAVTTEKAEEQPATVKAEVVNTEVKAPEA ALKDSEVEAALSLKNIKNIDGKYYYVNEDGSHKENFAITVNGQLLYFGKDGALTSSST YSFTPGTTNIVDGFSINNRAYDSSEASFELIDGYLTADSWYRPASIIKDGVTWQASTA EDFRPLLMAWWPNVDTQVNYLNYMSKVFNLDAKYSSTDKQETLKVAAKDIQIKIEQKI QAEKSTQWLRETISAFVKTQPQWNKETENYSKGGGEDHLQGGALLYVNDSRTPWANSD YRRLNRTATNQTGTIDKSILDEQSDPNHMGGFDFLLANDVDLSNPVVQAEQLNQIHYL MNWGSIVMGDKDANFDGIRVDAVDNVDADMLQLYTNYFREYYGVNKSEANALAHISVL EAWSLNDNHYNDKTDGAALAMENKQRLALLFSLAKPIKERTPAVSPLYNNTFNTTQRD EKTDWINKDGSKAYNEDGTVKQSTIGKYNEKYGDASGNYVFIRAHDNNVQDIIAEIIK KEINPKSDGFTITDAEMKQAFEIYNKDMLSSDKKYTLNNIPAAYAVMLQNMETITRVY YGDLYTDDGHYMETKSPYYDTIVNLMKSRIKYVSGGQAQRSYWLPTDGKMDNSDVELY RTNEVYTSVRYGKDIMTANDTEGSKYSRTSGQVTLVANNPKLNLDQSAKLNVEMGKIH ANQKYRALIVGTADGIKNFTSDADAIAAGYVKETDSNGVLTFGANDIKGYETFDMSGF VAVWVPVGASDNQDIRVAPSTEAKKEGELTLKATEAYDSQLIYEGFSNFQTIPDGSDP SVYTNRKIAENVDLFKSWGVTSFEMAPQFVSADDGTFLDSVIQNGYAFADRYDLAMSK NNKYGSKEDLRDALKALHKAGIQAIADWVPDQIYQLPGKEVVTATRTDGAGRKIADAI IDHSLYVANSKSSGKDYQAKYGGEFLAELKAKYPEMFKVNMISTGKPIDDSVKLKQWK AEYFNGTNVLERGVGYVLSDEATGKYFTVTKEGNFIPLQLTGKEKVITGFSSDGKGIT YFGTSGTQAKSAFVTFNGNTYYFDARGHMVTNSEYSPNGKDVYRFLPNGIMLSNAFYI DANGNTYLYNSKGQMYKGGYTKFDVSETDKDGKESKVVKFRYFTNEGVMAKGVTVIDG FTQYFGEDGFQAKDKLVTFKGKTYYFDAHTGNGIKDTWRNINGKWYYFDANGVAATGA QVINGQKLYFNEDGSQVKGGVVKNADGTYSKYKEGFGELVTNEFFTTDGNVWYYAGAN GKTVTGAQVINGQHLYFNADGSQVKGGVVKNADGTYSKYNASTGERLTNEFFTTGDNN WYYIGANGKSVTGEVKIGDDTYFFAKDGKQVKGQTVSAGNGRISYYYGDSGKRAVSTW IEIQPGVYVYFDKNGLAYPPRVLN (SEQ ID NO: 40)

Exemplary GTF peptides are shown in Tables 17-19.

Table 17 Catalytic Domain Peptides of GTF
DANFDSIRVDAVDNVDADLLQI (SEQ ID NO: 25)
PLDKRSGLNPLIHNSLVDREVDDRE (SEQ ID NO: 26)

Table 18 Glucan-binding Domain Peptides of GTF
TGAQTIKGQKLYFKANGQQVKG (SEQ ID NO: 23)
DGKLRYYDANSGDQAFNKSV (SEQ ID NO: 27)

Table 19 Surface Domain Peptide of GTF
QWNGESEKPYDDHL (SEQ ID NO: 28)

Diepitopic peptide constructs, which induce protective antibody to both S. mutans GbpB, and GTF of *mutans streptococci* are made using known methods. One peptide is drawn from the GbpB sequence and the other peptide is drawn from the GTF sequence. Useful GTF sequences are described in U.S. Patent No. 5,686,075 and U.S. Patent Application 09/290,049. The multiepitopic sequences are placed on a multiple antigenic peptide (MAP) backbone, expressed recombinantly or in an attenuated expression vector or by other methods known in the art. For example, diepitopic immunogenic and vaccine compositions include S. *mutans* GbpB peptide KSNAATSYINAIINSKSVSD (SEQ ID NO: 1) combined with *S. sobrinus* GTF-B residues 1303 to 1324; TGAQTIKGQKLYFKANGQQVKG (SEQ ID NO: 23) or *S mutans* GTF-B residues 442 to 462; DANFDSIRVDAVDNVDADLLQ (SEQ ID NO: 24).

The peptides are directly linked to one another or are separated by intervening residues (*e.g.*, one or more lysines). The compositions optionally contain an immunogenicity-enhancing agent, such as a bacterially-derived adjuvant. For example, a GbpB or GTF peptide is conjugated to a known protein, (such as tetanus toxoid) or a carrier (such as a synthetic polymer carrier) to give a macromolecular structure to the composition, which enhances immunogenicity. The compositions contain at least two different peptides, and the peptides are synthesized and covalently attached to a peptidyl core matrix to yield a macromolecule with a high density of peptides in a single structure. Each peptide in such a structure comprises a GbpB peptide, which is of sufficient length to raise an immune response in a mammal to which it is administered. The composition optionally contains a plurality of copies of a GbpB or GTF peptide. Synthetic peptide vaccine design was carried out using a MAP construct using known methods, *e.g.*, Tam *et al.*, PNAS USA 85:5409-5413 (1988).

A peptidyl core matrix contains of amino acids such as lysine, arginine and histidine. In particular, at least 2 peptides are synthesized on a core matrix of at least one lysine to yield a macromolecular vaccine composition. Particularly, at least 2 peptides are synthesized on a core matrix of 3 lysines. In another example, the vaccine composition is made by covalently attaching 4 peptides to a core matrix of 3 lysines yielding a radially branched peptide with

four dendritic arms. The four peptides present can be the same or different. Such multiepitopic peptide constructs induced enhanced immune responses. Moreover, the combination of sequences from several strains into a synthetic or recombinant multi-epitopic construct increases the protective potential of subunit vaccines for dental caries.

Vaccine Formulations

Suitable peptide(s) are incorporated into a microparticle or microsphere, *e.g.*, a PLGA (poly(lactide-co-glycolide) adjuvant) microparticle, for improved delivery and immune response. Different particles or spheres have different release profles depending on properties, such as polymer material, pore size, total particle/sphere size, and degradation kinetics. Such bioadhesive microparticles can facilitate primary and secondary mucosal antibody formation. Microparticles prepared from the biodegradable and biocompatible polymers, the poly(lactide-co-glycolides) or (PLG), have been shown to be effective adjuvants for a number of antigens. Moreover, PLG microparticles can control the rate of release of entrapped antigens and therefore, offer potential for the development of single-dose vaccines. To prepare single-dose vaccines, microparticles with different antigen release rates are combined as a single formulation to mimic the timing of the administration of booster doses of vaccine. Adjuvants can also be entrapped within the microparticles or, alternatively, adjuvants can be co-administered.

Other examples of suitable microparticles or microspheres, which can be mixed with or loaded with the proteins, peptides, or antibodies described herein, include, but are not limited to, poly(sebacic anhydride) (PSA) microspheres (Berkland *et al.*, *J. Controlled Release* vol. 24 (2003)); poly(ethylene glycol)/polylactide nano-particles (Caliceti *et al.*, *J. Controlled Release* vol. 24 (2003)); oligo(poly(ethylene glycol) fumarate) (OPF) (Holland *et al., J. Controlled Release* vol. 24 (2003)).

Other suitable biocompatible, biodegradable polymers include, for example, poly(lactides), poly(glycolides), poly(lactide-co-glycolides), poly(lactic acid)s, poly(glycolic acid)s, poly(lactic acid-co-glycolic acid)s, polycaprolactone, polycarbonates, polyesteramides, polyanhydrides, poly(amino acids), polyorthoesters, polycyanoacrylates, poly(p-dioxanone), poly(alkylene oxalate)s, biodegradable polyurethanes, blends and copolymers thereof.

Further, the terminal functionalities of the polymer can be modified. For example, polyesters can be blocked, unblocked or a blend of blocked and unblocked polymers. A blocked polymer is as classically defined in the art, specifically having blocked carboxyl end

groups. Generally, the blocking group is derived from the initiator of the polymerization and is typically an alkyl group. An unblocked polymer is as classically defined in the art, specifically having free carboxyl end groups.

Acceptable molecular weights for polymers used in this invention can be determined by a person of ordinary skill in the art taking into consideration factors such as the desired polymer degradation rate, physical properties such as mechanical strength, and rate of dissolution of polymer in solvent. Typically, an acceptable range of molecular weights is of about 2,000 Daltons to about 2,000,000 Daltons. In a preferred embodiment, the polymer is a biodegradable polymer or copolymer. In a more preferred embodiment, the polymer is a poly(lactide-co-glycolide) (hereinafter "PLGA") with a lactide:glycolide ratio of about 1:1 and a molecular weight of about 5,000 Daltons to about 70,000 Daltons. In an even more preferred embodiment, the molecular weight of the PLGA used in the present invention has a molecular weight of about 6,000 to about 31,000 Daltons.

The microparticles or microspheres are 0.25 - 6.0 microns in dimension. Suitable microparticles are 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, or 6.0 microns.

A sustained release composition of the invention contains from about 0.01% (w/w) to about 50% (w/w) of protein, peptide, or antibody incorporated into particles. The amount of such particles used will vary depending upon the desired effect of the protein, peptide, or antibody, the planned release levels, the times at which protein, peptide, or antibody should be released, and the time span over which the protein, peptide, or antibody will be released. A preferred range of particle loading is between about 0.1% (w/w) to about 30% (w/w) protein, peptide, or antibody to particles. A more preferred range of protein, peptide, or antibody to particle loading is between about 0.1% (w/w) to about 20% (w/w) particles. The most preferred loading of the particles is about 15% (w/w).

The sustained release composition of this invention can be formed into many shapes such as a film, a pellet, a cylinder, a disc or a microparticle A microparticle, as defined herein, comprises a polymeric component having a diameter of less than about one millimeter and having protein-, peptide-, or antibody-loaded particles dispersed therein. A microparticle can have a spherical, non-spherical or irregular shape. It is preferred that a microparticle be a microsphere. Typically, the microparticle will be of a size suitable for injection. A preferred size range for microparticles is from about 1 to about 180 microns in diameter.

A suitable polymer solution contains between about 1% (w/w) and about 30% (w/w) of a suitable biocompatible polymer, wherein the biocompatible polymer is typically dissolved in a suitable polymer solvent. Preferably, a polymer solution contains about 2% (w/v) to about 20% (w/v) polymer. A polymer solution containing 5% to about 10% (w/w) polymer is most preferred.

The method for forming a composition for modulating the release of a biologically active agent from a biodegradable polymer is further described in U.S. Pat. No. 5,656,297 to Bernstein et al. One suitable method for forming a sustained release composition from a polymer solution is the solvent evaporation method described in U.S. Pat. No. 3,737,337, issued to Schnoring et al., U.S. Pat. No. 3,523,906, issued to Vranchen et al., U.S. Pat. No. 3,691,090, issued to Kitajima et al., or U.S. Pat. No. 4,389,330, issued to Tice et al. Another method for forming sustained release microparticles from a polymer solution is described in U.S. Pat. No. 5,019,400, issued to Gombotz et al. This method of microsphere formation, as compared to other methods, such as phase separation, additionally reduces the amount of protein, peptide, or antibody required to produce a sustained release composition with a specific protein, peptide, or antibody content.

The proteins, peptides, or antibodies described herein can also be conjugated to polymers, such as *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer (Nan *et al., J. Controlled Release* vol. 24 (2003); polyvinylpyrrolidone (PVP) (Souza *et al., J. Controlled Release* vol. 24 (2003)); branched poly(L-glutamic acid) attached to poly(amidoamine) (PAMAM) dendrimer or polyethyleneimine (PEI) cores (Tansey *et al., J. Controlled Release* vol. 24 (2003)); or bacterial polysaccharide or lipopolysaccharide (LPS) (*see e.g.,* Frosch, M. in "Vaccine Delivery Strategies").

Additionally, other ways of enhancing immune responses to mucosally applied peptides (antigens) include use of mucosal adjuvants such as detoxified versions of tetanus toxin (*e.g.* tetanus toxin Fragment C), cholera toxin or *E. coli* heat-labile toxins (Smith *et al., Infect. Immunity* 69(8):4767-4773 (2002)). Other immunostimulatory adjuvants include LPS derivatives, saponins, CpG oligonucleotides, and cytokines.

Peptides are formulated with a physiologically acceptable medium. The physiological medium may include, but is not limited to, water, buffered saline, polyols (*e.g.*, glycerol, propylene glycol, liquid polyethylene glycol) and dextrose solutions. The optimum concentration of the active ingredient(s) in the chosen medium can be determined empirically, according to procedures well known to in the art, and will depend on the ultimate

pharmaceutical formulation desired. Methods of introduction of exogenous peptides at the site of treatment include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, oral, sublingual, intraocular, rectal and intranasal. Other suitable methods of introduction can also include rechargeable or biodegradable devices and slow release polymeric devices. The compositions of this invention can also be administered as part of a combinatorial therapy with other agents.

Peptides are administered at an intravenous dosage of approximately 1 to 100 μmoles of the polypeptide per kg of body weight per day. Administration is typically parenteral. For example, the peptides are administered intravenously, subcutaneously, intramuscularly, intraperitoneally, orally, or intranasally.

Antibody Production

The peptides of the invention are used to raise antibodies or to elicit an immune response. The term "antibody" as used herein refers to immunoglobulin molecules and immunogenically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that specifically binds an antigen. A molecule that specifically binds to a peptide of the invention is a molecule that binds to that peptide or a fragment thereof, but does not substantially bind other molecules in a sample, *e.g.*, a biological sample, which naturally contains the peptide. Examples of immunogenically active portions of immunoglobulin molecules include F(ab) and $F(ab')_2$ fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal, monoclonal, and transgenic antibodies that bind to a peptide of the invention. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of a peptide of the invention. A monoclonal antibody composition thus typically displays a single binding affinity for a particular peptide of the invention with which it immunoreacts.

Polyclonal antibodies are prepared as described above by immunizing a suitable subject with a desired immunogen, *e.g.*, the whole glucan binding protein-B, a peptide of the invention or fragment thereof. The antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized peptide or protein. If desired, the antibody molecules directed against the peptide can be isolated from the mammal (*e.g.*, from the blood) and further purified by well-known techniques, such as protein A chromatography to obtain the IgG

fraction. Also, antibodies can be isolated from the yolks of immunized chicken eggs (IgY) (Svendsen *et al., Lab. Anim. Sci.* 45:89-93 (1995)) or transgenic antibodies can be prepared in plants or other hosts and extracted for human use (Ma *et al., Eur. J. Immol.*, 24:131-138 (1994)). At an appropriate time after immunization, *e.g.*, when the antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein, *Nature*, 256:495-497 (1975), the human B cell hybridoma technique (Kozbor *et al., Immunol. Today*, 4:72 (1983)), the EBV-hybridoma technique (Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96 (1985)) or trioma techniques. The technology for producing hybridomas is well known (see generally Ausubel, *et al.* (Eds.), *Current Protocols in Immunology*, John Wiley & Sons, Inc., New York, NY (2001)). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with an immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds a peptide of the invention.

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating a monoclonal antibody to a peptide of the invention (see, *e.g.*, *Current Protocols in Immunology, supra*; Galfre *et al.*, Nature, 266:55052 (1977); R.H. Kenneth, in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); and Lerner, *Yale J. Biol. Med.*, 54:387-402 (1981)). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods that also would be useful.

Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal antibody to a peptide of the invention can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (*e.g.*, an antibody phage display library) with the peptide to thereby isolate immunoglobulin library members that bind the peptide. Kits for generating and screening phage display libraries are commercially available (*e.g.*, the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *Surf*ZAP™ Phage Display Kit, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, U.S. Patent No. 5,223,409; PCT Publication No. WO 92/18619; PCT Publication No. WO 91/17271; PCT Publication No. WO 92/20791; PCT Publication No. WO 92/15679; PCT Publication No. WO 93/01288; PCT Publication No. WO 92/01047;

PCT Publication No. WO 92/09690; PCT Publication No. WO 90/02809; Fuchs *et al.*, Bio/*Technology*, 9:1370-1372 (1991); Hay *et al.*, *Hum. Antibod. Hybridomas*, 3:81-85 (1992); Huse *et al.*, *Science*, 246:1275-1281 (1989); Griffiths *et al.*, *EMBO J.*, 12:725-734 (1993).

Additionally, recombinant antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art. The invention also is intended to cover human antibodies. Methods for production, isolation purification and use are known to those skilled in the art using standard methodologies.

Active immunization with *Streptococcus mutans* has been shown to induce protection against experimental dental caries. Protection results from continuous secretion of salivary antibody to Gbp-B. Also contemplated by the present application is the use of the peptides described herein for conferring passive immunity to a mammal by administration of antibodies directed to the peptides of the invention using the methods described by Smith *et. al., Infect. and Immun.* 69(5):3135-3142 (2001). Passive immunity and protection from *Streptococcus mutans* can result from administration of antibodies specific to Gbp-B peptides (*e.g.*, SEQ ID NOS. 1-22) as described herein. Routes for administration include intravenous, intranasal, topical, and dietary, including the inclusion of the antibody in mouthwash, toothpaste or chewing gum. The administration of Gbp-B peptide antibodies can have an immunotherapeutic efficacy for dental caries by interfering with the accumulation of *S. mutans* in the biofilm and the subsequent events that cause dental caries.

The present invention further relates to a method of provoking an immune response to glucan binding protein or to glucosyltransferase in a mammal by administering an immunogenic or vaccine composition of the invention. Preferably, the immune response results in interference with glucan binding in the biofilm in mammals after administration of the vaccine composition. Alternatively, the immune response results in interference with the enzymatic activity of glucosyltransferase in mammals. The immune response elicited by the compositions and methods of the invention can be humoral or systemic; for example, the immune response can be a mucosal response. The immune response elicited by the method of the present invention results in reduction of the colonization or accumulation of mutans

streptococcal strains in the mammal to which the vaccine or immunogenic composition is administered.

The compositions of the present invention are administered to any mammal in which the prevention and/or reduction of dental caries is desired. Suitable mammals include primates, humans, cats, dogs, mice, rats and other mammals in which it is desirable to inhibit dental caries. The present invention provides a vaccine that is useful for preventing, halting or reducing the progression of dental caries in a mammal to which the vaccine is administered.

In the method of the present invention of provoking an immune response to GbpB, mammals in which an immune response to GbpB is desired are given the vaccine or immunogenic compositions described herein. The compositions can be included in a formulation, which is administered to an individual being treated; such a formulation can also include a physiologically compatible carrier (*e.g.*, a physiological buffer), stabilizers, flavorants, adjuvants and other components. The vaccine can be administered by a variety of routes (*e.g.*, parenterally, mucosally, intranasally, intraocularly, intravenously, rectally, orally) and the components of the formulation will be selected accordingly. The amount to be administered and the frequency of administration can be determined empirically and will take into consideration the age and size of the mammal being treated and the stage of the dental caries disease (*e.g.*, prior to colonization of *mutans streptococci*, soon after colonization of *mutans streptococci* or in later stages of colonization).

EXAMPLES

Example 1: Identification of Immunogenic Regions

Peptides presented in conjunction with class II MHC molecules were derived from GbpB that has been processed in the phagosome of the antigen processing cell. The peptides bind to MHC molecules on the surface of these cells in a linear fashion. The binding was determined by the interaction of the peptide's amino acid side chains with the binding pockets in the MHC molecule. The characteristics of peptides that are likely to bind to a given MHC were directly deduced from pooled sequencing data of MHC alleles, resulting in an estimated binding probability. Thus, in order to identify potential B cell epitopes within GbpB sequence, which can be used for design of subunit vaccines, a matrix-based algorithm for epitope prediction (EpiMatrix; EpiVax, Inc, Providence, RI) was used to search the primary amino acid sequence of GbpB for known MHC class II binding motifs.

The motif-matching algorithms analyzed the GbpB sequence against each MHC class II allele to indicate regions of sequence that contain clusters of binding motifs. Those sequences with sufficiently high estimated binding probabilities (EBP) were used to predict MHC ligands. FIG. 1 illustrates regions of predicted epitopes as the number of motif matches associated with a given sequence. Four regions within the expressed protein sequence were identified which had at least 6 matches within the N terminal region of the expressed protein sequence. One of these regions, beginning at residue 16, fell within the 27 residue signal peptide. Three other regions in the N terminal third of the molecule began at residue 62, residue 90 and residue 121. One peptide in the C terminal region, beginning with residue 369, had 5 matches. Independent analysis of other sets of known alleles also identified these as regions with higher estimated binding probabilities. These latter analyses also showed the 10-mer region following residue 322 to have more binding potential than predicted from the results shown in FIG. 1. Table 1 indicates the peptide sequences that were identified by this method.

Table 1

Amino Acid Sequence of GbpB Peptide	SEQ ID NO:
113 KSNAATSYINAIINSKSVSD 132	SEQ ID NO: 1
KHKLITIQGQVSALQTQQAG	SEQ ID NO: 2
306 TATEAQPSASSASTAAVAAN 325	SEQ ID NO: 3
6 LSAVLVSGVTLSSATTLSAV 25	SEQ ID NO: 4
16 LSSATTLSAVKADDFDAQIA 35	SEQ ID NO: 5
33 QIASQDSKINNLTAQQQAAQ 52	SEQ ID NO: 6
37 QDSKINNLTAQQQAAQAQVN 56	SEQ ID NO: 7
48 QQAAQAQVNTIQGQVSALQT 67	SEQ ID NO: 8
52 QAQVNTIQGQVSALQTQQAE 71	SEQ ID NO: 9
88 QQIQTLSSKIVARNESLKQQ 107	SEQ ID NO: 10
117 ATSYINAIINSKSVSDAINR 136	SEQ ID NO: 11
137 VSAIREVVSANEKMLQQQEQ 156	SEQ ID NO: 12
174 TVAANQETIAQNTNALNTQQ 193	SEQ ID NO: 13
194 AQLEAAQLNLQAELTTAQDQ 213	SEQ ID NO: 14
214 KATLVAQKAAAEEAARQAAA 233	SEQ ID NO: 15
248 ALQEQAAQAQVAANNNTQAT 267	SEQ ID NO: 16
289 TEQSAAQAVNNSDQESTTAT 308	SEQ ID NO: 17
311 QPSASSASTAAVAANTSSAN 330	SEQ ID NO: 18
349 GNYWGNGGQWAASAAAAGYR 368	SEQ ID NO: 19
365 AGYRVGSTPSAGAVAVWNDG 384	SEQ ID NO: 20
383 DGGYGHVAYVTGVQGGQIQV 402	SEQ ID NO: 21
403 QEANYAGNQSIGNYRGWFNP 422	SEQ ID NO: 22

Peptide Constructs

MAP constructs of three 20-mer peptides (SYI, QGQ, SAS), which included the predicted binding epitopes following residues 62, 121 and 322 were synthesized using the following peptides:

SYI (KSNAATSYINAIINSKSVSD; GbpB residues 113-132) (SEQ ID NO: 1)

QGQ (QAQVNTIQGQVSALQTQQAE; GbpB residues 52-71) (SEQ ID NO: 9); and SAS (TATEAQPSASSASTAAVAAN; residues 306-325) (SEQ ID NO: 3).

The constructs (SYI, QGQ and SAS; SEQ ID NOs. 1, 3 and 9) were selected for synthesis and further analysis based on the estimated high MHC Class II binding probability identified in the matrix-based approach described above. Peptides were synthesized (Applied Diagnostics, Foster City, CA) using the stepwise solid phase method of Merrifield R.B., *J. Amer. Chem. Soc.* 85:2149-2154 (1963) on a core matrix of lysines to yield macromolecules with four peptides per molecule, after the method of Tam *et al.*, *PNAS USA* 85:5409-5413 (1988). Synthesis was successful with two of the three peptides (SYI and QGQ). Purity (>90%) was assessed using HPLC, amino acid analysis, and molecular weight determination by mass spectrometry.

Glucan Binding Protein (GbpB)

GbpB was purified from *S. mutans* strain SJr by ion exchange chromatography on MONO-Q HR 5/5 (Pharmacia) in the presence of urea. Bacteria were cultivated in sucrose-free defined medium as previously described by Navarre and Schneewind in *Mol. Microbiol. 14*:115-121 (1994). GbpB prepared in this manner migrates as a single protein band in SDS-polyacrylamide gel electrophoresis.

ELISA

Serum IgG and salivary IgA antibodies were tested by enzyme-linked immunosorbent assay (ELISA). Polystyrene microtiter plates (Flow Laboratories) were coated with 2.5 mg/ml of SYI or QGQ or 0.5 mg/ml of *S. mutans* GbpB. Antibody activity was then measured by incubation with 1:400 and 1:4000 dilutions of sera, or 1:4 dilutions of saliva. Plates were then developed for IgG antibody with rabbit anti-rat IgG, followed in sequence by alkaline phosphatase goat anti-rabbit IgG (Biosource Inc.) and p-nitrophenylphosphate (Sigma Chemical Co., St. Louis, MO). A mouse monoclonal reagent to rat α chain (Zymed, South San Francisco, CA) was used with biotinylated goat anti-mouse IgG (Zymed), followed by avidin-alkaline phosphatase (ICN Biomedicals, Inc., Auroa, OH), followed by p-nitrophenylphosphate to reveal levels of salivary IgA antibody to peptides. Reactivity was recorded as absorbance (A405 nm) in a micro plate reader (Biotek Instruments, Winooski, VT). Data are reported as ELISA units (EU), which were calculated relative to the levels of appropriate reference sera or salivas from Sprague Dawley rats twice immunized with the

respective peptide construct. Dilutions of sera producing an A405 nm of approximately 1.0 were considered 100 EU for serum IgG antibody measurements. Dilutions of saliva producing an A405nm of approximately 0.8 were considered 100 EU for salivary IgA antibody.

Immunogenicity of Peptides

Sprague Dawley CD strain 45 day-old female rats (Charles River Laboratories, Wilmington, MA) were used for injection. Four groups of 6 rats/group were injected subcutaneously in the vicinity of the salivary glands with 50 μg each of SYI or QGQ peptide constructs, or 10 μg of GbpB, or sham-immunized with buffer alone. The initial injection included complete Freund adjuvant (CFA; Difco Laboratories, Detroit, MI); one subsequent injection 21 days later included incomplete FA. Animals were bled prior to injection and 14 days after the second injection. In this experiment, rats were first momentarily anesthetized with a gas mixture of 50% carbon dioxide and 50% oxygen, and then anesthetized by intra-peritoneal injection of a mixture (0.65 ml/kg) of 3 parts ketamine (Ketaset, 100 mg/ml, Fort Dodge Lab, Ft. Dodge, Iowa) and seven parts xylazine (Rompun, 20 mg/ml, Bayer Corp., Shawnee Mission, Kansas). Saliva secretion was stimulated by subcutaneous injection of 0.6 ml carbachol (containing 0.1 mg/ml in saline; Sigma Chemical Co., St. Louis, MO) per kilogram of rat weight. After fluid collection, rats were injected subcutaneously first with 0.1 ml/kg of atropine sulfate (0.4 mg/ml; American Pharmaceutical Partners, Inc., Los Angeles, CA) and then with yohimbine (yobine, 2.0 mg/ml; Lloyd Laboratories, Shenandoah, IO) at a volume equal to 1.4 times that used for anesthesia. Sera from coagulated and centrifuged blood were stored frozen at -20°C until measurement of antibody activity. Serum taken thirty-five days after the first injection was analyzed in ELISA for serum IgG antibody levels to each peptide construct and to GbpB (FIG. 2).

All rats injected with the QGQ peptide responded with high levels of serum antibody to the QGQ peptide, whereas no significant response to QGQ epitopes were seen in sham immunized rats or rats injected with SYI. Interestingly, the sera from two of the four rats injected with GbpB protein also reacted with QGQ.

All rats injected with the SYI peptide also demonstrated elevated levels of serum IgG antibody to the inciting SYI MAP peptide construct, in contrast to sham- or QGQ-injected rats. Again, serum IgG from one of the four rats injected with the parent GbpB protein also showed a significant reaction with the SYI peptide.

All sera were also evaluated in ELISA using plates coated with GbpB. Rats from SYI (6/6) or QGQ (4/6) peptide-injected groups reacted with the parent GbpB protein. Although the levels of serum IgG antibody from peptide-injected rats that were reactive with GbpB did not achieve levels from protein-injected rats, the overall response in the SYI-injected rats to native GbpB epitopes was significant. Taken together, these results supported the immunogenicity of these peptides predicted using the bioinformatics approach. Furthermore, they also suggested that the linear epitope(s) found especially on the SYI peptide construct were shared with those on the intact parent GbpB protein.

Protective Immunity

Peptides and multiple-epitope peptide constructs were tested in an art-recognized rat model for human dental caries. The SYI peptide was selected to test this assumption since this peptide induced more consistent immune responses reactive with GbpB than did the QGQ peptide.

Two groups (n=13/group) of 25 day-old Sprague-Dawley female rats were singly caged. Rats were subcutaneously (sc) injected in the salivary gland vicinity with 50 μg of SYI MAP peptide construct or phosphate buffered saline (control animals). Antigen was incorporated with complete Freund's adjuvant (CFA). Nine days later, rats were reinjected with PBS or with SYI at the same dose in incomplete FA. Six days after the second injection, blood and saliva was collected under anesthesia described above. About fifteen days after the second injection, rats were placed in tubs (6 rats/ tub), given diet 2000, and orally infected with approximately 10^8 *S. mutans* SJ32 for 3 consecutive days. Rats were again singly caged after the infection protocol was completed and continued on diet 2000 for the duration of the experiment. Blood and saliva were collected 78 days after initial infection, followed by sacrifice. In preparation for the scoring of dental caries, rat skulls were defleshed by dermaphagic beetles, followed by a rinse with 70% ethanol.

Sera collected at the end of the 78 day infection period were analyzed for IgG and IgA antibody to both the peptide construct (FIG. 3) and to GbpB (FIG. 4). As expected, immunization with the peptide induced serum antibody in both isotypes to the inciting SYI peptide. Also, consistent with the previous experiment, SYI immunization also induced IgG antibody to intact GbpB in all rats, although some rats did not demonstrate serum IgA antibody levels to GbpB, at least at the dilutions tested. Saliva was collected prior to infection and at the end of the experiment was analyzed in ELISA for IgA antibody to SYI

and GbpB (Table 2). Several (5/13) SYI-immunized rats demonstrated induction of salivary IgA antibody to both the peptide and the intact protein at either time point, although group levels were not significantly different under the conditions of measurement.

Table 2

Group	Test Antigen	Mean EU	SE
Sham-immunized	GbpB	7.1	5.1
SYI-immunized	GbpB	31.1	16.5
Sham-immunized	SYI	3.6	2.4
SYI-immunized	SYI	12.5	5.9

The protective response of the SYI immunization was evaluated by systematic swabbing of molar teeth for *S. mutans* infection (FIG. 5) and measurement of caries on molar surfaces (FIG. 6).

Bacterial Recoveries

The mutans streptococcal flora was assessed at 70 days after infection. After systematic swabbing of teeth, sonication, and plating appropriate dilutions on *mitis salivarius* agar (MS; total streptococci), and MS agar with 0.2 mg/ml streptomycin sulfate (MSS; Streptococcus mutans strain SJr), plates were incubated for 48 hours at 37°C in 90% N_2, 10% CO_2. *S. mutans* colony forming units (CFU) were then enumerated microscopically on MSS agar.

Caries Assessment

The extent and depth of carious lesions in all rat molar teeth (caries score) were microscopically evaluated by standard methods. Caries scores were determined separately on smooth and on occlusal dental surfaces. Thus, measurements of protective influence of immunization support the conservation of at least one epitope on SYI capable of inducing a caries-protective response in this model. The mean levels of infecting (streptomycin tolerant) *S. mutans* SJr recovered from SYI-immunized groups were lower than sham-immunized group recoveries both eight and 65 days after infection was initiated, although these differences did not achieve statistical significance at the $p < 0.05$ level because of the variation in bacterial recoveries. The trend in the infection data was supported by measurements of dental caries. Caries scores on smooth (buccal) and occlusal surfaces as well as total caries scores of SYI-peptide immunized rats were significantly lower than those of sham-immunized and infected rats (FIG. 6).

Example 2: Diepitopic Immunization Studies

GTF and glucan binding protein B (GbpB) from *mutans streptococci* have each been implicated in the molecular pathogenesis of dental caries caused by these organisms. Native GTF and GbpB, as well as synthetic peptides derived from each protein, have been shown to induce protective immune responses to infection with cariogenic mutans streptococci in experimental models.

Two diepitopic synthetic peptide constructs were synthesized in a MAP format. Both peptides contained SYI, a 20-mer sequence from GbpB that bioinformatic analyses indicated was similar in sequence to an MHC class II binding peptide. One diepitopic peptide (SYI-CAT) also contained a 22-mer sequence from the catalytic domain of GTF. The other diepitopic construct (SYI-GLU) contained a 22-mer sequence from the glucan binding domain of GTF.

Diepitopic and monoepitopic MAP constructs were synthesized by AnaSpec, Inc. (San Jose, CA). Eight groups of Sprague-Dawley rats (n=4-8/group) were initially injected subcutaneously with one the following, together with complete Freund adjuvant: (1) buffer alone, (2) MAP-CAT, (3) MAP-GLU, (4) MAP-SYI, (5) MAP-CATGTF-SYIGbpB, (6) MAP-GLUGTF-SYIGbpB, (7) *S. mutans* GbpB, or (8) *S. sobrinus* GTF. On day 21, the 8 groups were again injected with the same contents, except the second injections substituted incomplete Freund adjuvant. Animals were then bled and salivated on days 42 and 63. Sera

were tested for antibody activity against peptides and proteins using an alkaline phosphatase enzyme-linked immunosorbent assay (ELISA). Antibody levels were compared using one and two way ANOVA, followed by Dunn's multiple comparison test.

Sera from blood taken 42 days after the second injection were examined for IgG antibody activity against constituent peptides or native proteins. The serum IgG response to GLU was similar whether SYI-GLU or GLU alone was used for injection. In contrast, SYI-CAT induced an IgG response to CAT that was significantly higher than that induced by CAT alone. Both diepitopic peptide constructs induced IgG antibody that reacted with GTF and GbpB native proteins. Sera from SYI-CAT-immunized animals reacted with GTF to a significantly greater degree than SYI-GLU. These results indicate that diepitopic synthetic peptides, especially SYI-CAT, induce an immune response that provides a broader range of protective antibody epitopes in a subunit dental caries vaccine. Furthermore, these results indicate that the combination of SYI with CAT potentiated the immune response to this important GTF catalytic domain.

Sequences Used in Diepitopic Immunization Studies:

1. GTF-derived catalytic (CAT) peptide: DANFDSIRVDAVDNVDADLLQI (SEQ ID NO:25)

2. GTF-derived glucan binding (GLU) peptide: TGAQTIKGQKLYFKANGQQVKG (SEQ ID NO:23)

3. GbpB-derived MHC class II (SYI) peptide: KSNAATSYINAIINSKSVSD (SEQ ID NO:1)

4. Diepitopic SYI-CAT peptide, two copies of each in multiple antigenic peptide (MAP) format: KSNAATSYINAIINSKSVSD (SEQ ID NO:1) – DANFDSIRVDAVDNVDADLLQI (SEQ ID NO:25)

5. Diepitopic SYI-GLU peptide, two copies of each in multiple antigenic peptide (MAP) format: KSNAATSYINAIINSKSVSD (SEQ ID NO:1) – TGAQTIKGQKLYFKANGQQVKG (SEQ ID NO:23)

Results of Diepitopic Immunization Studies:

Sera were tested at a 1:200 dilution in groups of 4-7 rats.

Both diepitopic constructs induce significant antibody to Gbp-B (Table 3).

Table 3: Serum IgG responses to GBP-b (glucan binding protein B)

Group	Serum IgG antibody to GbpB Mean ± SE
Sham	0.027 ± 0.014
SYI-CAT	0.783 ± 0.268
SYI-GLU	0.847 ± 0.186
SYI	0.599 ± 0.201
CAT	0.029 ± 0.009
GLU	0.022 ± 0.009
GluB	1.838 ± 0.052

The SYI-GLU diepitopic construct enhances anti-peptide (SYI) and anti-glucan binding protein responses (FIG. 7). Rats immunized with either diepitopic construct develop antibody to the parent GbpB protein (FIG. 8).

The SYI-CAT diepitopic construct significantly enhances the anti-peptide (CATI) responses over the mono-epitopic MAP (FIG. 10). Only the SYI-CAT diepitopic construct induced significant IgG antibody to the parent GTF protein in all rats (FIG. 11), and thus was the most efficient stimulus for antibody to both virulence antigens. Furthermore, the SYI-CAT diepitopic construct alone induced a significant serum IgG immune response to GTF in all animals (Table 4).

Table 4: Serum IgG responses to GTF

Group	Serum IgG antibody to GTF Mean + SE
Sham	0.114 ± 0.029
SYI-CAT	0.955 ± 0.200
SYI-GLU	0.083± 0.018
SYI	0.073 ± 0.018
CAT	0.088 ± 0.018
GLU	0.108 ± 0.031
GTF	1.728 ± 0.098

The SYI-CAT construct induces significant levels of serum IgG antibody to both GbpB and GTF virulence antigens of *mutans streptococci*. In addition, the diepitopic construct enhanced the immune response to the CAT epitopes over that observed when monoepitopic CAT construct is used. Thus the SYI-CAT construct reduces the pathogenicity

of *Streptococcus mutans* by inhibiting enzymatic activity (glucan formation) and inhibiting activity of glucan binding protein B.

All references cited herein are incorporated by reference in their entirety. While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

Example 3: Immunogenicity of Glutamine-Rich Peptides from *S. mutans* GbpB

Two peptides with MHC class II binding characteristics were evaluated for their ability to induce serum and salivary antibodies. Two 20-mer MAP peptides were synthesized: QGQ from the N-terminal region (residues 52-71) and QAA from the central region (residues 252-270). In the first of 3 experiments, groups of 42 day-old rats (5-8/group) were either sham-immunized or injected with 60 μg QAA, twice 21 days apart. Rats were bled and salivated on days 22, 63, and 83. In experiment 2, weanling rats (15/group) were sham-immunized or injected with 60 μg QAA, or injected with 6 μg GbpB, twice 13 days apart. After bleeding and salivation, rats were infected with *S. mutans* SJr and caries were measured 67 days later. In experiment 3, weanling rats (15/group) were sham-immunized, or injected with 60 μg QGQ or injected with 6 μg GbpB, twice 14 days apart; fluids were collected 8 days later. Antibody was measured by ELISA.

In experiments 1 and 2, sera from groups injected with QAA contained significant levels of IgG antibody to QAA ($p<0.01$). 3/8 rats (exp. 1) and 3/14 rats (exp. 2) had detectable antibody to GbpB. Salivary responses were delayed and seen in a minority of rats. No caries protection was observed. In contrast, QGQ (exp. 3) induced a rapid serum IgG response to QGQ. In addition, significant levels of serum ($p<0.02$) and salivary ($p<0.03$) antibody to GbpB were detected in QGQ-immunized rats. Therefore, epitope(s) in the QGQ sequence are superior to those in QAA for induction of systemic and mucosal antibody to GbpB.

Example 4: Caries Protection by Intranasal Immunization with *S. mutans* GbpB Peptide

SYI, a 20mer peptide from *S. mutans* glucan binding protein B (GbpB), has MHC class II binding characteristics. One group of weanling Sprague Dawley rats (n=13) were immunized subcutaneously with adjuvant when rats were 25 and 34 days old (sham). A

second group (n=13) was immunized intranasally with 60 μg SYI, mixed (immunization days 25 and 32) with or loaded (immunization day 39) in PLGA microparticles (IN-SYI). All intranasal immunizations were given with 5 μg cholera toxin (CT). Nasal washes and salivas were collected on day 40. Mucosal IgA antibody to SYI< GbpB and CT was measured by ELISA. Beginning on day 45 all rats were orally infected with 10^8 *S. mutans* SJr for three consecutive days. On day 98 rats were sacrificed, saliva and asal washes collected, and molars scored for dental caries.

All rats given SYI intranasally had demonstrable IgA antibody to CT in salivas and nasal washes prior to infection ($p<0.001$). Salivary IgA antibody to SYI could be detected in most peptide-imunized rats before infection. Subsequent studies revealed that SYI-loaded PLGA gave far higher salivary IgA responses to SYI and GbpB than did SYI mixed with PLGA. IN immunization with SYI resulted in significantly lower occlusal ($p<0.01$) and total ($p<0.03$) caries. Thus, protective immune response by salivary antibody was produced by mucosal application of a GbpB subunit vaccine.

Example 5: MHC Class II Alleles Bound to Glucosyltransferase Select Immunogenic Peptides

In order to select highly immunogenic peptides, 2 different quantitative matrices to predict MHC Class II binding regions in *S. sobrinus* GTF sequence. Fifty-one Class II alleles were assessed for binding to GTF allowing identification of promiscuous binding regions. Regions of GTF with defined functional relevance were also considered. Twenty candidate peptides (20mer) were selected, synthesized and tested for reactivity with serum IgG antibody obtained from rats hyperimmunized with GTF pool, (n=3) or naïve control animals (n=3) by ELISA. Additionally, lymph node and spleen cells from GTF immunized once in CGA (n=2) or from a naïve rat were restimulated with peptides *in vitro* to determine proliferative T cell responses.

Several regions of GTF were identified which were predicted to bind the majority of Class II alleles analyzed. A number of binding regions were conserved between the different GTFs of *mutans streptococci*. Serum antibody from GTF-immunized rats, but not naïve animals, bound some of these peptides. In particular, peptides encompassing amino acids 478-497 and 847-866 demonstrated exceptional reactivity with anti-GTF sera, and also stimulated *in vitro* proliferation of lymph node and spleen cell cultures. ELISA analysis of

human sera containing antibody to GTF also demonstrated reactivity against some of the same peptide sequences.

Table 20 Peptides of *S. sobrinus* GTF-I
NNHVSIVEAWSDNDTPYLHDD (SEQ ID NO: 42)
VVIANNVDKFVSWGITDFEM (SEQ ID NO: 43)

Table 21 Peptides of *S. sobrinus* GTF-U
VTDSEANALAHISILEAWSL (SEQ ID NO: 44)
NNDADYTNKKIAENADFFKK (SEQ ID NO: 45)

5